

We claim:

1. A method of stimulating a population of undifferentiated mesodermally derived cells to undergo at least one of hematopoiesis and vascular growth; comprising:
  - (a) selecting a compound that is functionally equivalent to a gene product expressed in an embryo's extraembryonic tissue;
  - (b) causing the compound to access the cells, so as to stimulate the cells to undergo at least one hematopoiesis and vascular growth.
2. A method according to claim 1, wherein the compound is a secreted protein.
3. A method according to claim 1, wherein the compound is a hedgehog compound.
4. A method according to claim 3, wherein the compound is an agonist of a hedgehog protein binding receptor.
5. A method according to claim 4, wherein the hedgehog protein binding receptor is patched.
6. A method according to claim 1, wherein the compound causes enriched expression of *Gli*.
7. A method according to claim 3, wherein the hedgehog compound is selected from the group consisting of Indian hedgehog, Desert hedgehog and Sonic hedgehog compound.
8. A method according to claim 3, wherein the compound is an Indian hedgehog compound,

9. A method according to claim 1, wherein the compound is a first compound derived from a first gene product and is capable of acting synergistically with a second compound that is derived from a second gene product expressed in the extraembryonic tissue, so as to enhance the stimulation of at least one of hematopoiesis and vascular growth.

10. A method according to claim 9, wherein the second compound is a functional equivalent of a TGF- $\beta$  family member.

11. A method according to claim 9, wherein the TGF- $\beta$  is selected from the group consisting of BMP-2, BMP-4, BMP-6 and BMP-7.

12. A method according to claim 1, wherein the extraembryonic tissue is visceral endoderm.

13. A method according to claim 1, wherein the extraembryonic tissue is yolk sac mesoderm.

14. A method according to claim 1, further comprising the step of maintaining the cell population *in vitro* in a culture medium such that step (b) includes providing the compound in the culture medium.

15. A method according to claim 14, wherein the cells are a population of hematopoietic stem cells.

16. A method according to claim 15, wherein the hematopoietic stem cells are selected from the group consisting of cord blood cells, fetal peripheral blood cells and fetal liver cells.

17. A method according to claim 15, wherein the hematopoietic stem cells are adult bone marrow cells.

18. A method according to claim 14, wherein the cells are progenitor cells obtained from an adult human.

19. A method according to claim 14, wherein the cells are precursor cells from an adult human capable of vascular growth when stimulated by the compound.

20. A method according to claim 14, wherein the cells constitute embryonic tissue.

21. A method according to claim 20, wherein the cells constitute an embryonic explant culture.

22. A method according to claim 21, wherein the embryonic explant culture is a blastocyst.

23. A method according to claim 1, wherein the cells are *in vivo* hematopoietic stem cells within the bone marrow of an animal.

24. A method according to claim 1, wherein the cells are hematopoietic stem cells present in the animal and are selected from the group of hematopoietic cells found in at least one of bone marrow, cord blood cells, fetal peripheral blood cells and fetal liver cells.

25. A method according to claim 24, further comprising causing the compound to access the stem cells, by administering an effective dose of the compound to the animal by any of oral, intradermal, subcutaneous, transmucosal, intramuscular or intravenous routes.

09021660.021098

26. A method according to claim 2, wherein the compound is functionally equivalent to a protein from the bone marrow morphogenic protein (BMP) family.

27. A method of treating developmental errors in vascular growth or hematopoiesis in an embryo in utero, comprising:

- (a) selecting an effective dose of a compound that is functionally equivalent to a gene product expressed in an extraembryonic tissue; and
- (b) causing the compound to access a population of embryonic cells *in vivo*, so as to stimulate the cells to undergo at least one of hematopoiesis and vascular growth.

28. A method according to claim 27, wherein the compound is an agonist of a hedgehog protein-receptor.

29. A method according to claim 27, wherein the compound is a hedgehog protein.

30. A method according to claim 27, wherein the compound is a first compound capable of acting synergistically with a second compound that is derived from a second gene product expressed in the extraembryonic tissue, so as to enhance the stimulation of hematopoiesis in embryonic cells.

31. A method of treating a subject suffering from an abnormal number of erythroid cells, comprising:

- (a) selecting an effective dose of a compound that is functionally equivalent to a gene product expressed in an extraembryonic tissue; and
- (b) causing the compound to access a population of hematopoietic stem cells over an effective time so as to modulate the number of cells undergoing at least one of proliferation or hematopoiesis.

32. A method according to claim 31, wherein the compound is an agonist of a hedgehog protein-receptor and the hematopoietic stem cells are stimulated to undergo one of proliferation or hematopoiesis.

5 33. A method according to claim 32, wherein the compound is a hedgehog protein.

34. A method according to claim 31, wherein the compound is a first compound capable of acting synergistically with a second compound that is derived from a second gene product expressed in the extraembryonic tissue, so as to enhance the stimulation of hematopoiesis in hematopoietic stem cells.

10 35. A method according to claim 31, wherein the compound is an antagonist of a hedgehog protein and the hematopoietic stem cells are inhibited from undergoing one of proliferation or hematopoiesis.

15 36. A method according to claim 31, wherein the abnormal number of erythroid cells is an abnormally low number of erythroid cells characterized by an anemia in the subject, the anemia being selected from the group consisting of idiopathic aplastic anemia, constitutional aplastic anemia, secondary forms of aplastic anemia, myelodysplastic anemic, viral induced chronic anemia, chronic inflammatory disease induced anemia, cancer induced anemia, chronic anemia induced by organ failure, thrombocytopenia and drug induced anemia.

20 37. A method according to claim 31, wherein the abnormal number of erythroid cells causes a disease selected from the group consisting of polycythemia vera and erythroleukemia.

25 38. A method of treating a subject suffering from an ischemia in tissues, comprising:  
(a) selecting an effective dose of a compound that is functionally equivalent to a gene product expressed in an extraembryonic tissue; and

(b) administering the compound to the ischemic site over an effective time so as to stimulate vascular growth within the ischemic tissues.

39. A method according to claim 37, wherein the ischemia is myocardial ischemia.

40. A method according to claim 38, wherein the compound is an agonist of a hedgehog protein-receptor.

41. A method according to claim 40, wherein the compound is a hedgehog protein.

42. A method according to claim 39, wherein the compound is a first compound that is capable of acting synergistically with a second compound that is derived from a second gene product expressed in the extraembryonic tissue, so as to enhance the stimulation of vascular growth.

43. A method of treating abnormally enhanced vascular growth in a subject, comprising:

(a) selecting an effective dose of a hedgehog compound capable of inhibiting the activity of a gene product expressed in an extraembryonic tissue; and

(b) administering the compound to the subject over an effective time so as to inhibit abnormally enhanced vascular growth.

44. An *in vitro* assay for determining the activity of a compound capable of modulating hematopoiesis or vascular growth, comprising:

(a) selecting a population of cells from a tissue derived from a fertilized egg of a mammal, wherein the population of cells is deficient in blood formation as detectable by the absence of a predetermined marker; and

(b) adding an agent to the population of cells so as to reverse the deficiency.

45. An assay according to claim 44, wherein the population of cells is a blastocyst.

46. An assay according to claim 44, wherein the population of cells is an epiblast, the epiblast characterized by an absence of visceral endoderm and isolated from the mammal prior to appearance of blood islands as detectable by histological staining.

47. An assay according to claim 44, wherein the population of cells is a portion of an epiblast, the portion being the anterior section, the epiblast isolated from the mammal prior to the appearance of blood islands in the anterior portion as detectable by histological staining.

48. An assay according to claim 47, wherein the mammal is a transgenic mouse.

49. An assay according to claim 48, wherein the transgenic mouse is formed by a modification of the mouse genome.

50. An assay according to claim 49, wherein the modification is selected from one of a random insertion of a DNA sequence, a targeted insertion of a DNA sequence, a targeted deletion of a DNA sequence and a targeted replacement of a DNA sequence.

51. An assay according to claim 50, wherein the transgenic mouse is homozygous for the modification.

52. An assay for determining the activity of a compound capable of modulating hematopoiesis or vascular growth, comprising:

- (a) selecting a first transgenic animal carrying a marker:  $\epsilon$ -globin hybrid gene; wherein the  $\epsilon$ -globin gene is capable of expression at least up to 15.5 dpc.;
- (b) mating the first transgenic animal to a second animal that is similarly transgenic;
- (c) isolating an embryo from the mating during the gestation period; and

(d) determining the effect of the compound on the stimulation of hematopoiesis and vascular growth in the isolated embryo by measuring marker expression.

53. A method according to claim 52, wherein a further step preceding step (d) comprises; separating embryonic tissue from extraembryonic tissue.

54. An assay according to claim 52, wherein the marker is a histochemical marker selected from the group consisting of LacZ, alkaline phosphatase, green fluorescent protein and derivatives.

55. An assay according to claim 52, wherein the embryo is a mouse embryo, and the mouse embryo is isolated between approximately 6.0-12.0 dpc.

56. An assay according to claim 52, wherein the embryo is a mouse embryo and the mouse embryo is isolated between approximately 3.5-3.75 dpc.

lan 57238

Add B<sub>2</sub> 7

add B<sub>2</sub>

add E<sub>6</sub>

09021650 09972060

10

15

20